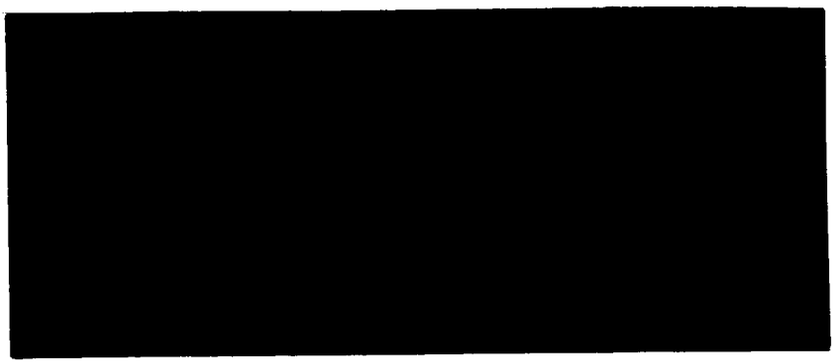

MAGNA CORPORATION

Research and Development Division

1001 SOUTH EAST STREET • ANAHEIM, CALIFORNIA

QUARTERLY PROGRESS REPORT NO. 2
July 1 to September 30, 1963

RESEARCH ON APPLIED BIOELECTROCHEMISTRY
Contract NASw-623

MAGNA CORPORATION

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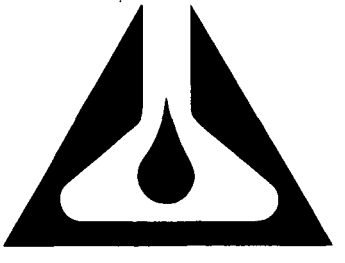

339/7014/T6
October 1963

QUARTERLY PROGRESS REPORT NO. 2

July 1 to September 30, 1963

t: RESEARCH ON APPLIED BIOELECTROCHEMISTRY Quarterly...

(NASA Contract NASw-623)

(NASA CR-52606; 339/7014/T6; 714-772-1261) OTS:

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1.0 PURPOSE

The purpose of work undertaken in this effort is to find environmental conditions and microbial life which are most favorable to bioelectrochemical utilization of human wastes in space vehicles. The principal function of this program is obtaining power from bioelectrochemical cells using human wastes. A secondary function of this program is conversion of the waste materials to chemicals that can be reused in maintenance of the space vehicle and its occupants.

This effort has been subdivided into three major tasks. The first, a literature search and preliminary selection of organisms and enzymes which are attractive (a) for production of electrochemically active chemicals from human wastes, and (b) for conversion of waste materials, is completed. The experimental effort is divided between essentially biological functions and essentially electrochemical functions, into two tasks. The biological task is converned with screening and characterization of microorganisms and enzymes with respect to conditions which predispose to their most effective conversion of wastes to electroactive chemicals and to otherwise useful chemicals. The electrochemical task has as its purpose the evaluation of biological electrodes with respect to optimizing and defining electrochemical parameters for effective utilization of the waste materials, primarily with respect to production of power. A further function of the electrochemical task is the preparation of biological electrodes having the biological material immobilized at the electrode surface.

Other programs which relate directly to the present work are being undertaken by Marquardt Corp. under Contract NASw-654 and Aeronutronic Corp. under Contract NASw-655. These programs are concerned with development and fundamental research in bioelectrochemistry of human wastes, respectively. Magna Corporation, under Contract DA 36-039 SC-90866, is performing research on biochemical fuel cells. Further, Magna has a contract with the Department of the Navy (NObs 84243) to study biochemically promoted power sources.

2.0 ABSTRACT

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Two methods have been used for calculating the theoretical electrochemical energy available from urine and feces used as fuels. The first, based upon heats of combustion, yields a maximum energy of 265 watt hours per man-day's output. The second, based upon converting urine nitrogen to ammonia and feces polysaccharides to hydrogen and using these as electrochemical fuels with oxygen, yields a maximum energy of 73.6 watt hours per man-day's output.

Using anaerobic growth of Bacillus pasteurii in urine or production of ammonia via urea hydrolysis, virtually 100% utilization of urea and 90-95% conversion to ammonia was shown. Under similar conditions, use of additional urea in the urine decreased efficiency of conversion of urea to ammonia. Temperature studies showed optimum ureolytic activity at 30-35°C.

Study of mixed urine and feces has been directed to (1) examining effects of feces on B. pasteurii activity in urine and (2) the production of hydrogen and ammonia from urine-feces mixtures. In case (1), some inhibition was seen even at 4% feces in urine. In case (2), use of a primary sewage plant culture at low pH gave no hydrogen, although methane production was absent.

Continuous culture of B. pasteurii in urine has been initiated to establish optimum conditions for urea hydrolysis.

A cell for the study of electrochemical parameters of B. pasteurii culture in urine over long time periods has been designed and fabricated. Use of this cell will begin during the coming quarter.

AUTHOR

3.0 CONFERENCES

Conferences have been held at approximately monthly intervals between Magna Corp. and Marquardt Corp. personnel. The purpose of these conferences has been the coordination of efforts relating to Contracts NASw-623 and NASw-654. During July, personnel of Aeronutronic Division, working on their Contract NASw-655, visited Magna Corporation facilities to discuss mutual problems relating to their respective contracts. During the same month, Magna Corp. personnel visited Aeronutronic facilities for the same purpose.

A meeting was held at Magna Corp. on August 2, 1963 and attended by Dr. M. G. Del Duca, Dr. E. Cohn, H. Schwartz and P. Pomerantz, all of NASA, to discuss technical status of Contract NASw-623. A similar meeting was held at Magna Corp. on September 12, 1963, attended by M. Unger and P. Pomerantz of NASA. An important question discussed at these two meetings, but only partially resolved at the writing of this report, was the emphasis to be placed on work under the program on power generation vs. waste handling aspects. Mr. Unger proposed a meeting, of all concerned with the contract, to be held in November at which, among other business, this questions would be resolved.

4.0 FACTUAL DATA

4.1 Introduction

Research on applied bioelectrochemistry as it relates to the use of urine and feces has proceeded. Subsequent to searching the literature for organisms and enzymes most favorable for (a) bioelectrochemical production of power from human wastes and (b) bioelectrochemical conversion of human wastes to chemicals reusable in a closed environment, an experimental program was formulated and begun directed to qualification of optimum biological conditions and optimum electrochemical conditions for these two functions. The straightforward conversion of urine and feces to known electrochemically active intermediates is the route being taken for power production. The secondary problem, conversion of wastes to chemicals which are reusable in a closed environment, is being approached via application of electrochemical means to promoting waste degradation. Analysis of information on feces is presented which leads to the suggestion that power production from that component of human wastes should perhaps be the secondary consideration.

The work during this quarter has been directed primarily to utilization of Bacillus pasteurii with urine and urine-feces mixtures. Limitations on the rate at which feces, obtained in a collection program at Magna, can be prepared and composited have limited work with feces.

4.2 Power from Human Wastes

4.2.1 Theoretical Fuel Value

From the heats of combustion of the human wastes, urine and feces, a maximum value can be estimated for power that can be produced using these as fuels. Obviously, various limitations apply, however, the power is to be extracted, and the heats of combustion provide only a limiting value. Heats

of combustion are used because the most likely approach to producing power from urine and feces electrochemically will be to employ oxygen as the oxidant.

4.2.1.1 Urine

The major organic constituent of urine is urea. While urea production is dependent on a number of factors, the normal average quantity produced per day by one man is 22 g. Higher than normal urea production is encountered when high-protein diets are consumed; up to twice the normal urea output may be obtained under such conditions. Other significant organic materials in urine, again based on an average man-day, are amino acids, 2.5 g; creatinine, 1.5 g; and hippuric acid, 1.5 g. The following tabulation shows the contribution of each of these components to the heat of combustion of urine:

	Kcal	Watt Hours
Urea	58	68
Amino acids (taken as 1/2 alanine, 1/2 phenylalanine)	14	16
Creatinine	7.5	8.7
Hippuric acid	8.5	9.9
Total	88	102.6

Note that more than half the heat of combustion derives from the single component urea. Further, the relatively high figure quoted for amino acids is very approximate and would come from many more individual compounds than the two used for the estimate.

The heat of combustion of urine provides a guide to the maximum energy that might be obtained from it. Further consideration must be given to the processes for obtaining such energy and to the limitations which apply to these processes.

Practical considerations indicate that a single process, in terms of the controls that must be applied, would be most desirable for conversion of the

chemical energy of urine to electrical energy. Because the components of urine are not yet amenable to direct conversion in power-producing reactions, at least two processes must be considered: a chemical conversion step to produce an electrochemically active intermediate and the electrode reaction process. As presently conceived, both these processes would be combined. That is, the chemical transformation, effected through some form of biological catalysis, would be carried out in the presence of an electrode.

The production of more than one electrochemically active intermediate from urine would require the use of more than one electrode, unless all such intermediates could react at nearly the same electrode potential. While such a scheme would allow maximum conversion of chemical to electrical energy, it is desirable to minimize the number of processes employed. This then leads to the consideration that all components of urine be transformed to a single intermediate. However, no single process has been conceived that would accomplish this.

One of the most practical expedients is to concentrate on the most abundant component of urine and to maximize the efficiency of converting the chemical energy of that component to electrical energy. In urine, the most abundant component which can lead to an electrochemically active intermediate is urea.

Earlier, the normal average output of 22 g. urea per man-day was equated with approximately 68 watt hours based on heat of combustion data. Let us consider (1) how this heat of combustion can be converted to electrical power, and (2) how much of this energy might be practically obtained. Without considering a mechanism for this conversion (mechanism will be treated subsequently), urea may be hydrolyzed to ammonia and carbon dioxide:

$$H_2N\ CONH_2 + H_2O \longrightarrow 2\ NH_3 + CO_2$$

Ammonia is an electrochemically active intermediate, which can in turn be employed at a fuel cell anode:

$$2\ NH_3 \longrightarrow N_2 + 6\ H^+ + 6\ e^-$$

Assuming oxygen as the fuel cell oxidant, the following cell reaction may be taken as a goal and used for estimating efficiency:

$$2\ NH_3 + 3/2\ O_2 \longrightarrow N_2 + 3\ H_2O, E^\circ = 1.13\ V$$

Assuming further that essentially no losses occur in the conversion of urea to ammonia and that conversion of ammonia is not rate limiting, the theoretical energy available from 22 g. of urea is:

$$\frac{22}{60}\text{mole} \times 6\ \frac{\text{equiv.}}{\text{mole}} \times 26.8\ \frac{\text{amp hr.}}{\text{equiv.}} \times 1.13\ V = 66.6\ \text{watt hr.}$$

A reasonable voltage efficiency for this cell appears to be 70%, so that energy from urea in urine that may be available as electrical energy is 47 watt hours per man-day. Power available would be approximately 2 watts per man.

Similar exercises made with the minor organic components of urine must consider the electrochemically active intermediate and the electrode reaction that would be used: One approach to this is to assume a two-electron redox reaction for each of these components; the other is to assume that all the nitrogen could be converted to ammonia. Again, without regard to mechanism, estimates can be made for the energy of these compounds that might be converted to electrical energy. The first case would be encountered in almost any discrete enzyme-catalyzed redox reaction of these materials. The second case assumes essentially a three-electron reaction for the amino acids and hippuric acid, and a nine-electron change for creatinine. In the case of assuming a two-electron change for each component, an operating cell voltage must also be assumed.

The following tabulation gives estimates of the electrical energy and power made within the context of the foregoing assumption. A working cell voltage of 0.79 volt has been assumed for all cases. This value is 70% of the E° for the ammonia-oxygen reaction. It can be seen then, that urea is by far the most promising fuel component of urine.

Component	2-electron change		conversion to ammonia	
	Power, Watts/man-day	Energy, Watt hrs/man-day	Power, Watts/man-day	Energy, Watt hr/man-day
Amino acids (taken as 1/2 alanine, 1/2 phenylalanine)	0.04	0.91	0.06	1.4
Creatinine	0.02	0.55	0.1	2.5
Hippuric acid	0.015	0.36	0.02	0.53
TOTALS	0.075	1.82	0.18	4.4

4.2.1.2 Feces

The variability of feces is attested by the dearth of specific information on its composition. Thus, an estimate of heat of combustion by summing heats of combustion of components would be futile. An estimate in the literature, however, is that the caloric content of feces is 70-140 Kcal per man-day.[2] Accordingly, these correspond to 81.5 to 163 watt hours per man-day.

An analysis of feces as an electrochemical fuel is considerably more difficult than for urine, primarily because information on the composition of feces is limited. By applying certain assumptions, it is possible to arrive at useful numbers, however.

The first consideration must again be given to what electrochemically active intermediates can be produced from feces, the components of feces themselves being inactive. There are a large variety of compounds present in feces in only small quantities. Significant components of feces are the nitrogen content, lipid content, and polysaccharide content. The nitrogen content of dry feces has been estimated at 6%. Lipids amount to 15-25% of feces and polysaccharides to 15-30%. The polysaccharide content is perhaps most subject to variation. These values for the most part represent not only discrete compounds and undigested residues, but also the live and dead bacterial matter present in feces.

Electrochemically active intermediates which might be produced from these major components of feces are largely ammonia and hydrogen. A limited basis exists for conversion of nitrogenous compounds to ammonia. Somewhat firmer grounds exist for hypothesizing hydrogen production from polysaccharides. No biological methods are known that could even remotely be considered to convert the lipids to electrochemically active intermediates to any significant degree. Because of the large number of compounds present, discrete enzyme-catalyzed reactions have been rejected for their specificity.

For the purposes of discussion, the complete conversion of nitrogen to ammonia can be assumed, although attainment of such complete conversion seems unlikely. Based on an average daily output of 150 g. feces by an individual and an average solids content of 33%, average production of nitrogen is 3 g. Using assumptions described earlier for electrochemical utilization of ammonia results in an estimate that energy might be produced from feces on the foregoing basis at the rate of 14 watt hours per man day.

The polysaccharide content of feces may be considered as a hydrogen source through the sequence of first, conversion to monosaccharides, and second, fermentation of the monosaccharides. On the basis that all the polysaccharide could be converted to glucose, and from the best data for hydrogen production from glucose production (2.3 moles H_2 per mole glucose), the maximum polysaccharide content of feces can be equated with 0.19 mole hydrogen per man-day. Using 0.8 volt as a working cell voltage for a hydrogen-oxygen cell, this quantity of hydrogen represents 8.2 watt hours per man-day.

4.2.2 Utilization of Human Wastes as Electrochemical Fuels

Practical utilization of urine and feces as electrochemical fuels demands a mechanism for conversion of essentially inactive (electrochemically speaking) components of these wastes to electrochemically active intermediates. Such a mechanism is immediately available for conversion of urea in urine to ammonia:

the growth of B. pasteurii in urine results in catalysis. Bacterial production of hydrogen from fecal components is a potential mechanism for production of another electrochemically active intermediate. Further, bacterial production of ammonia from feces is still another. The work reported herein is devoted to aspects of these mechanisms.

Practical considerations lead also to the desirability of using urine and feces in mixtures. To this end, studies below are concerned, on the one hand, with the effect of feces on bacterial production of ammonia from urine. On the other hand, admixture of urine with feces is indicated for work on production of electrochemically active intermediates from fecal components, because feces, despite its high nitrogen content, is notoriously poor in nitrogen compounds that can contribute to bacterial action.

4.2.2.1 *Urine Studies*

1. Bacillus pasteurii culture

It was reported in the First Quarterly Progress Report[1] that the experimental screening program resulted in the selection of Bacillus pasteurii for subsequent studies. This organism was selected for its relatively rapid anaerobic growth in urine accompanied by efficient urea hydrolysis, and for its possible utility in aiding degradation of fecal protein.

Analytical methods for determining urea and ammonia in urine have been applied to B. pasteurii cultures. Using these techniques, the growth of this organism in urine has been shown to result in virtually 100% conversion of urea nitrogen to ammonia. The mole ratio of ammonia produced to urea utilized is approximately 1.8 - 1.9, demonstrating that only a small amount of ammonia is converted to cellular nitrogenous material.

During this report period, work on B. pasteurii was continued to establish optimum growth conditions under anaerobic conditions for application to subsequent electrochemicol and continuous culture studies.

An experiment was performed in which B. pasteurii was grown in urine in Warburg vessels to determine the quantity of free gas evolved. The over-all gas evolution was negligible, indicating that carbon dioxide and ammonia from urea hydrolysis are effectively kept in solution as ammonium carbonate. This permits the use of the analytical technique for ammonium ion. Warburg manometry may be useful, however, to measure dissolved carbon dioxide by terminal acidification of the cultures.

Temperature optimization studies with B. pasteurii grown in urine showed that urea utilization and ammonia production were inhibited at 25 C compared to growth at 30 C and 35 C. Essentially no difference was observed between cultures grown at the higher temperatures; therefore, 30 C was selected for subsequent studies.

An extensive study was made on the effect of adding extra urea to urine as a preliminary step in the use of concentrated urine. It is expected that this approach would enable work with reduced volumes of urine and also result in reduced electrical resistance of urine. Urine, containing 1.0% urea (10 mg urea/ml) was supplemented at various levels up to 3.9% urea. The results showed that urea utilization and urea concentration varied inversely. The urea utilization decreased from 96% to 58% over the range of urea concentration examined. At urea concentrations higher than 2.7%, urea utilization did not improve. Ammonia production did not increase at urea concentrations above 1.9%. Up to 1.9% urea, the mole ratio of ammonia produced to urea utilized was approximately 1.9; this ratio decreased to 1.7 at the highest urea concentration used. Based on the urea-ammonia stoichiometry, the data indicate that 1.9% urea appears to be the upper limit of tolerance, since efficiency decreases at higher concentrations. It is expected that by employing adaption procedures, B. pasteurii can be "trained" to tolerate higher levels of urea.

2. Continuous Culture of B. pasteurii

A preliminary continuous culture experiment with B. pasteurii growing in urine was initiated primarily for the purpose of learning important

operational parameters. The continuous culture was maintained for a period of five days. Decreasing retention time resulted in increased efficiency of urea conversion to ammonia. Ammonia production increased 50% and urea conversion increased 49% to a total utilization of 87% during a period of 116 hours. The mole ratio of ammonia produced to urea utilized stabilized at approximately 2.0 during the five day run. Before steady state was achieved and before maximum utilization of urea occurred, the experiment was discontinued due to the accumulation of a heavy precipitate on the walls of the growth chamber and overflow tube. The precipitate is probably largely calcium carbonate. To remedy these problems, the growth chamber is being redesigned with a larger base overflow tube and provisions are being made to periodically remove the precipitate with a scrubbing device.

3. Electrochemical Studies with B. pasteurii

A cell has been constructed for use in studies of the continuous production of power from urine for long periods of time. It was designed so that material and energy balance data can be obtained for various biochemical systems maintained under sterile conditions. In the first studies, Bacillus pasteurii will be utilized as the bacterial means of producing ammonia from urea, the ammonia then being used as an anodic fuel.

The cell design, shown in Figure 1, consists of six separate parts. The glass tubing has been fitted so as to minimize the use of rubber tubing. The anode and cathode half-cells are essentially identical, being designed in the shape of an L with an "O" ring joint (65/40) at the top and side. When assembled, they form a U cell having an internal diameter of 40 mm (1-9/16"). A membrane (cation, Nepton CR-61, Ionics Inc.) is clamped between the two half-cells to separate the electrolytes. The design is such that the membrane has a greater diameter than the electrodes, permitting the two electrodes to face each other directly. This arrangement minimizes the distortion of the current flow in the cell.

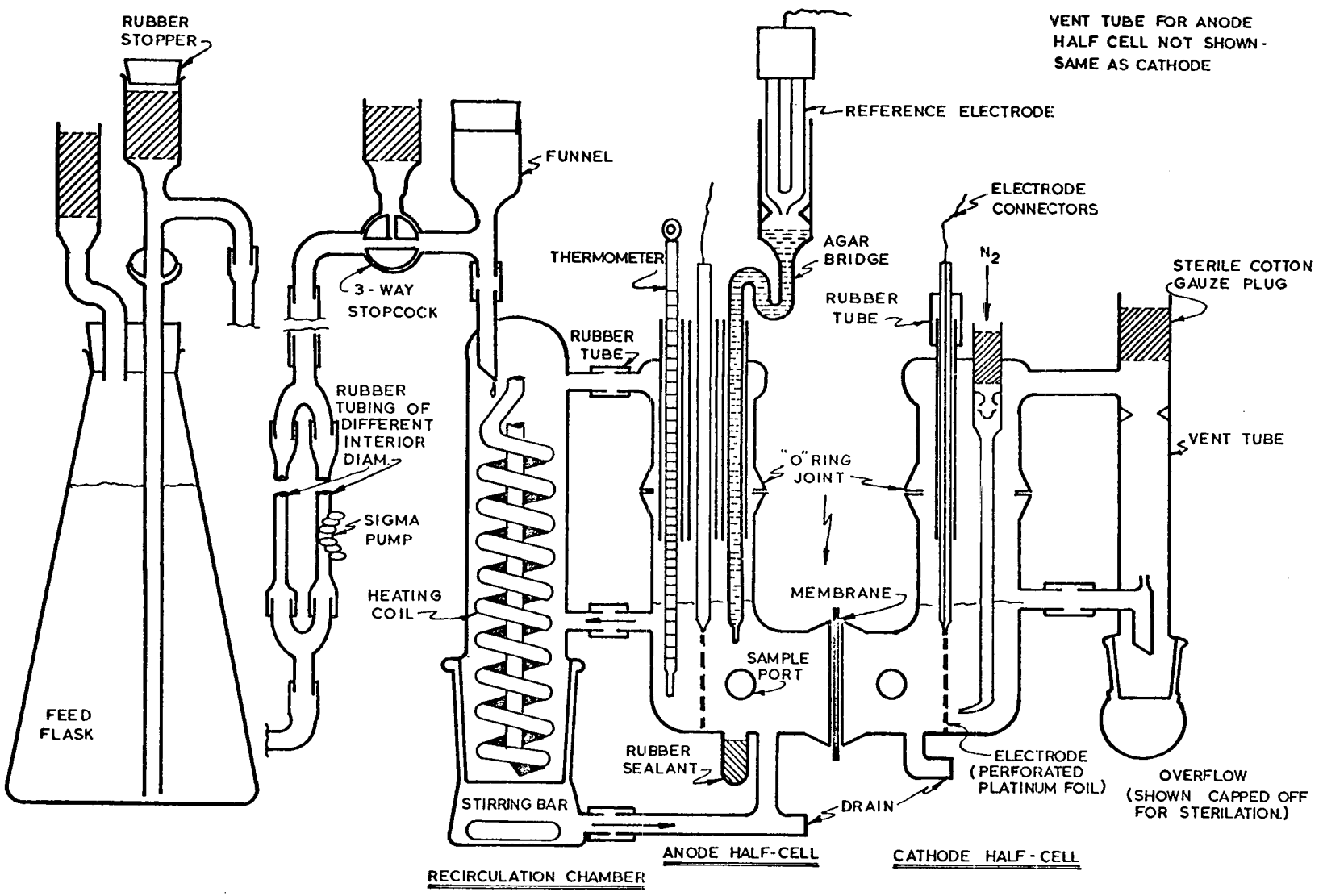

Figure 1: Cell for Electrochemical Evaluation of Urine Cultures

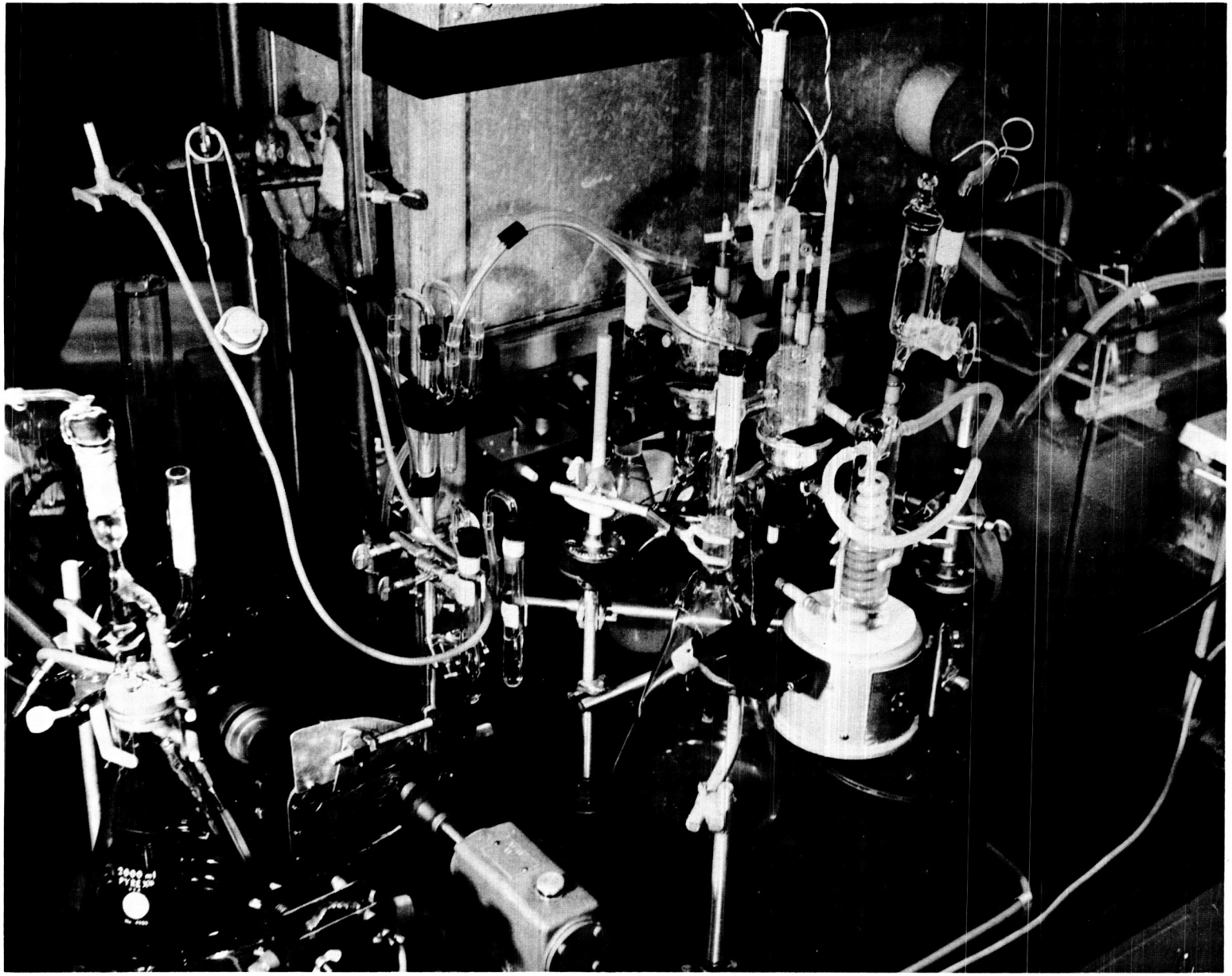

Figure 1-A Cell for Electrochemical Evaluation of Urine Cultures

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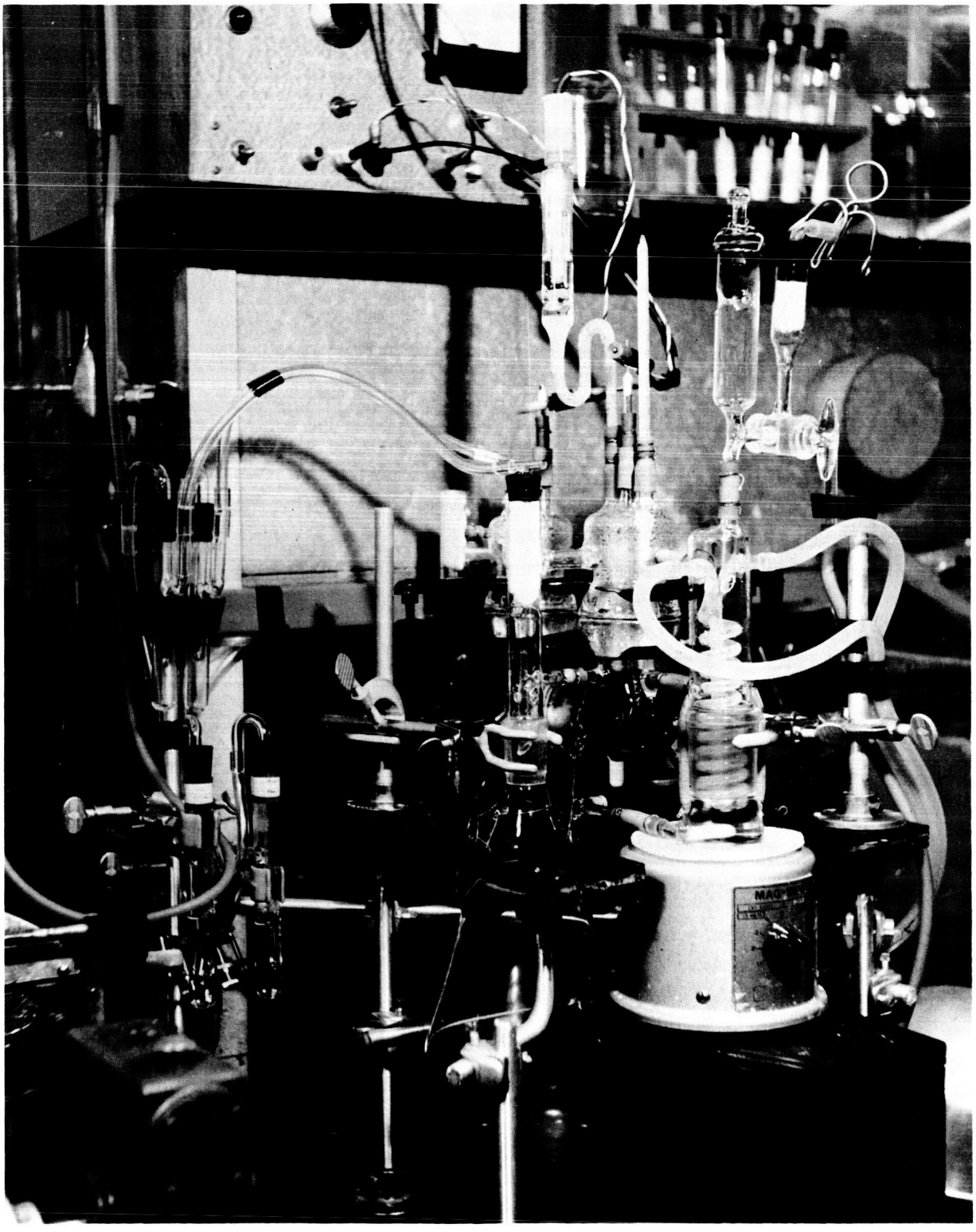

Figure 1-B Cell for Electrochemical Evaluation of Urine Cultures

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The test and auxiliary electrodes are perforated platinized platinum foil with a calculated area of 17.7 cm^2. They are spaced four inches apart. The internal resistance with a urine anolyte and a 1 M KCl catholyte has been measured at 24 ohms.

Since the reference electrode is a saturated calomel electrode which cannot be sterilized, a glass tube containing an agar solution is used to provide a bridge between the reference electrode and urine solution. In normal operation the capillary tip of the bridge is positioned near the surface of the anolyte so as to minimize disturbance at current distribution. During sterilization, the bridge is lowered until the capillary tip seats into a rubber sealant (Silastic RTV 731, Dow Corning), in a sealed glass tube at the bottom of the anode half-cell. This prevents the agar solution from draining out of the tube when it becomes liquid during sterilization.

The upper part or cap of each half-cell is clamped to the top "O" ring joint. In the anode half-cell, provision has been made for a thermometer, the test electrode, and the agar bridge, by using glass tubing through which each can be inserted. The cathode half-cell cap has openings for an auxiliary electrode, for purge gas (used to dilute and purge hydrogen as well as for agitation), and for liquid feed.

The vent tube in the cap of each half-cell is plugged with sterilized cotton. An overflow tube in the bottom of the half-cell is connected with rubber tubing to a continuation of the overflow in the vent tube. During operation, the flask which has been clamped to the vent tube to collect the overflow is thus cross-pressurized to the half-cell, and both are sealed from direct contact with the atmosphere by the sterile cotton plug.

The anode half-cell is heated and agitated by use of an agitation chamber consisting of two parts. The bottom part has a side outlet which can be connected to an inlet in the anode half-cell. The upper part is vented to the anode half-cell and contains a return line for recirculated anolyte. Fresh urine is fed into this recirculation chamber for mixing prior to contacting the electrode.

The recirculated anolyte passes over a glass coil through which water contained at a constant temperature is circulated. The total volume of anolyte is 215 ml (± 5 ml).

Fresh anolyte is fed to the cell by means of a Sigma pump equipped with a calibrated Zero-Max variable speed drive. The unit has been modified by inserting a speed reducer (Alling-Lander, 50/1) between the Zero Max and pump. A flow rate of from 1 to 42 ml/hr. can be obtained. The feed tube is connected to the agitation chamber through a three-way stopcock. A funnel is also provided as an integral part of the feed line for sterile liquids which may be required during operation. The feed tube connects to the feed flask by means of a ball joint so as to facilitate changing feed flasks without undue interruption of flow or chance of contamination.

Both half-cells are equipped with sample ports and drains stoppered with rubber serum caps. The cells have legs made of glass rod so that when assembled the unit stands level. In practice the assembly is wired to a board to which is affixed aluminum rods so that clamps can be used to take the weight of the overflow flasks.

The entire assembly, using sealed caps in place of overflow flasks, can be sterilized along with the rubber feed tubes. After sterilization, the board containing the assembled apparatus is mounted at a sufficient height to permit the overflow flasks to be properly placed below the cell assembly.

A constant temperature bath is employed to maintain the cell temperature at 30 ∓ 0.5°C.

4.2.2.2 Studies with Mixed Urine and Feces

1. Effects of feces on B. pasteurii culture

Although B. pasteurii grows well in urine alone, it is possible that supplementing urine with growth-limiting nutrients will improve growth and the rate of ammonia production. Urine contains many inorganic compounds, but

contains only marginal quantitites of organic carbonaceous material from which heterotrophic bacteria such as B. pasteurii can synthesize the bulk of their cell protoplasm. An obvious approach to providing utilizable, organic material in a closed ecology is the addition of feces to urine.

Work with feces was initiated by growing B. pasteurii in urine with 4, 20 and 50% fresh feces added. The highest urea utilization occurred in the culture with 4% feces, approximately 95% of the urea being utilized. In the 20% and 50% feces cultures, 89 and 80% of the urea, respectively, was consumed. In urine alone, 98% of the urea was consumed. Although not measured quantitatively, ammonia was detected in all cultures.

A subsequent experiment was performed to provide data on the mole ratio of ammonia produced to urea utilized. B. pasteurii was cultured in urine containing 1, 5 and 10% lyophilized feces. Approximately the same amount of ammonia was produced in the 1 and 5% feces cultures as in the control (urine alone) culture. Slightly reduced ammonia production occurred in the 10% feces culture. An evaluation of the urea determinations for this and the previous experiment indicate that feces interferes with the analysis; therefore, the urea data must be considered only approximate. The results, thus far, do not indicate that the addition of feces to urine enhances ammonia production; in fact, at the highest concentration examined, inhibition was observed. It is possible that feces contains toxic components that may be eliminated by suitable chemical or physical pre-treatment procedures. Another approach to reducing deleterious effects consists of acclimatizing B. pasteurii to feces by repeated subculture.

2. Production of H_2, NH_3 by Feces

There are two microbiological approaches which may be considered to produce hydrogen and ammonia from feces. The first employs a pure culture of a microbial species known to metabolize certain substrates to either hydrogen or ammonia. Hydrogen-producing species include Escherichia coli, Proteus vulgaris,

Veillonella gazogenes, Clostridium butyricum and Cl. tetanomorphum. Typical substrates are glucose, lactose, maltase, formate, certain organic acids and amino acids. Ammonia producing species include E. coli, Proteus vulgaris, Pseudomonas aeruginosa, and Cl. sporogenes. A wide variety of amino acids serve as substrates. A few proteolytic species are capable of hydrolizing proteins to amino acids as well.

The second approach consists of using enrichment procedures with a mixed sewage culture and/or indigenous fecal microflora. The object, in this case, is to provide a selective environment which favors the development and ultimate dominance of hydrogen or ammonia-producing organisms.

Hydrolytic enzymes such as cellulase, lipase, and some proteinases may contribute to bacterial action by degrading complex organic materials to simpler compounds, particularly with regard to the projected use of pure cultures. It is anticipated that enzymes would be employed to pretreat feces for subsequent bacterial metabolism rather than simultaneously with bacteria. An evaluation of the organic substrates found in feces suggests that enrichment techniques may be a more promising first approach than pure cultures. Feces contains virtually no low molecular weight carbohydrates and only marginal amounts of amino acids. A mixed culture offers the advantage of nutritional synergism whereby the more complex carbohydrate and amino acid polymers may be degraded to simpler, more available compounds for hydrogen and ammonia-producing organisms.

An attempt was made to produce hydrogen from feces by employing a mixed sewage culture and providing conditions favorable to hydrogen-producing bacteria. Treated sewage from a primary sewage disposal plant was inoculated into a medium consisting of lyophilized feces (sterilized) and urine. Urine was used to provide a nitrogen source (urea) for the bacteria since feces is poor in assimilable nitrogen. To encourage growth of hydrogen-producing bacteria and inhibit the methane bacteria, the pH of the culture was buffered at 5.5. The fermentation was partially successful in that no methane was produced and good digestion of organic solids and gasification occurred. However, only trace

amounts of hydrogen were detected. Subsequently, fermentations will be carried out with a recently acquired feces-adapted anaerobic mixture culture.

3. Enzymatic Treatment of Feces

Both cellulase and lipase were examined during this report period as potentially useful for pretreatment of feces. The objective is to facilitate action of selected bacteria on feces through breakdown of complex fecel components. Because the more attractive present approach with feces is the use of mixed cultures, the enzymatic pretreatment has been temporarily suspended. That is, the mixed bacterial cultures have the capability of nearly complete breakdown of fecal components and are, perhaps, better adapted to this breakdown than are the enzymes.

Cellulcse

Evaluation of commercially available cellulase showed that reducing sugars present in the preparation interfered with the colorimetric assay procedure for cellulose activity. The assay is dependent on production of reducing sugars from cellulose. Dialysis of the crude enzyme allowed removal of impurities to the extent that they could no longer be detected.

The influence of temperature on cellulase activity was studied at 30, 40, and 50°C. Representative data are shown in Figure 2. Over three hours of a 5-hour observation period, essentially no differences could be observed at the three temperatures; all data were within approximately 5%. During the last two hours, the 30°C run lagged the 40 and 50°C runs by approximately 10%. A sharp decrease in activity was seen during the first hour's observation. This break is believed due to accumulation of products such as cellobiose and glucose. Such inhibition can be overcome by retaining the reactive system (enzyme with cellulosic material) with a dialysis membrane through which low molecular weight products could diffuse readily.

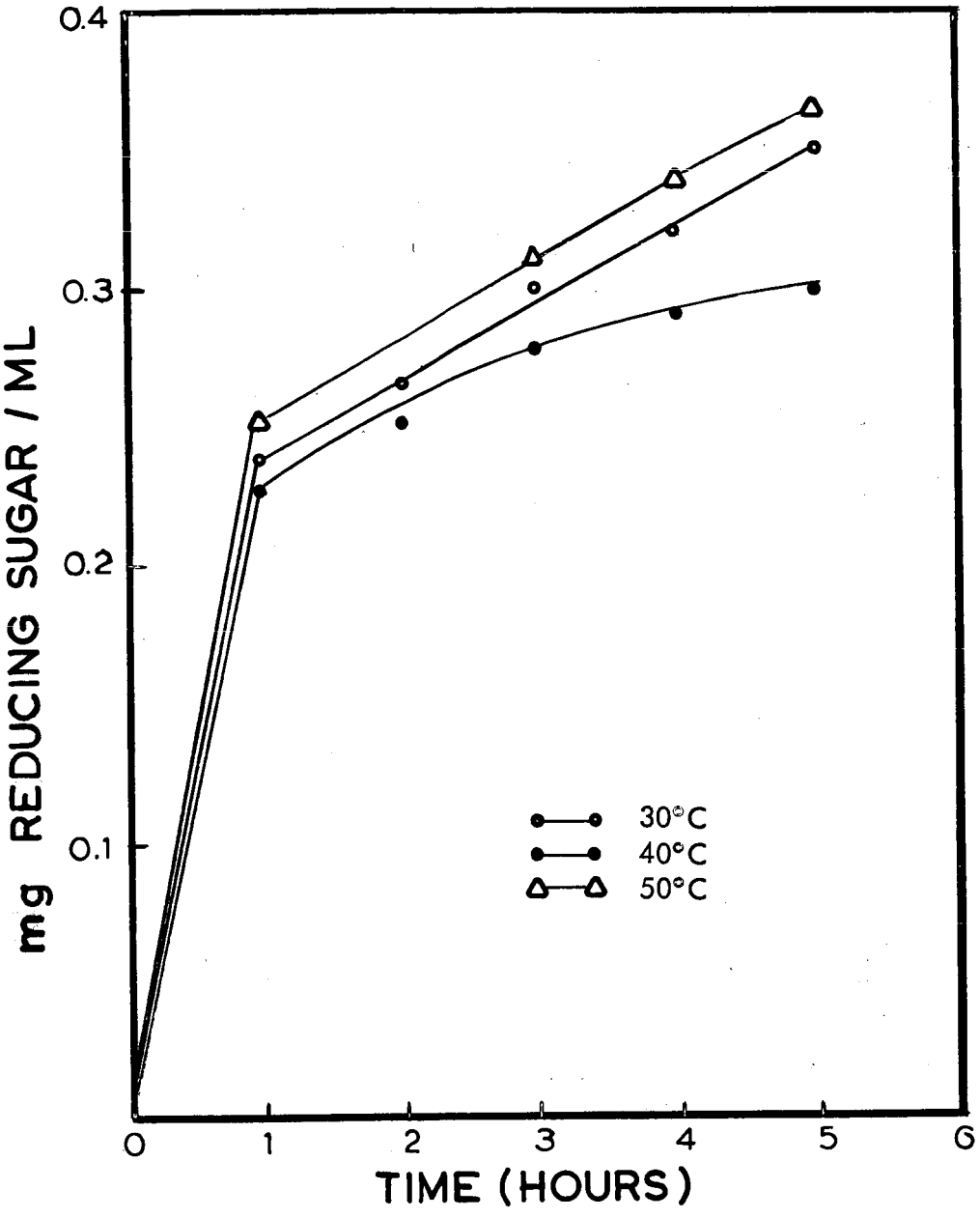

Figure 2

CELLULASE ACTIVITY WITH TEMPERATURE

Use of cellulase with feces is complicated by the brown color of feces interfering with the assay procedure. Attempts to decolorize feces with charcoal to facilitate assay were unsuccessful.

Lipase

Examination of a crude pancreatic lipase preparation showed good activity in an assay method using triacetin (glyceryl triacetate). Tests performed with the enzyme and a mixture of 20% feces in urine resulted in comparable activity as with triacetin. This is contrary to literature reports which suggest that feces inhibits lipase.[3] Data for lipase activity both with triacetin and with the urine-feces mixture are shown in Figure 3.

4.3 Waste Handling Aspects of Program

The maintenance of a closed ecology requires the recovery of all human wastes and the conversion of these materials to food, oxygen, and potable water. The objective of waste-recovery processes, whether physical, chemical, or biological, is to recover as much of the waste materials in a usable form as possible. Ideally, the waste process should convert waste materials into products which can be used in the food production system or recover the wastes in a form which can be directly used by man. Therefore, water and some inorganic compounds may be recovered and returned directly to man. Organic material may be converted to more oxidized compounds, such as H_2O, CO_2, NO_3^- and used in the food production process.

Although biological processes are considered to be more bulky and less reliable than most physical processes, they have the advantage of maintaining the maximum amount of waste materials in a biologically available form. This would allow the reutilization of the by-products of waste conversion processes directly by man or by any of the various related biological support systems, such as photosynthetic gas exchangers. The extent to which conventional microbiological waste treatment processes can be adapted to space

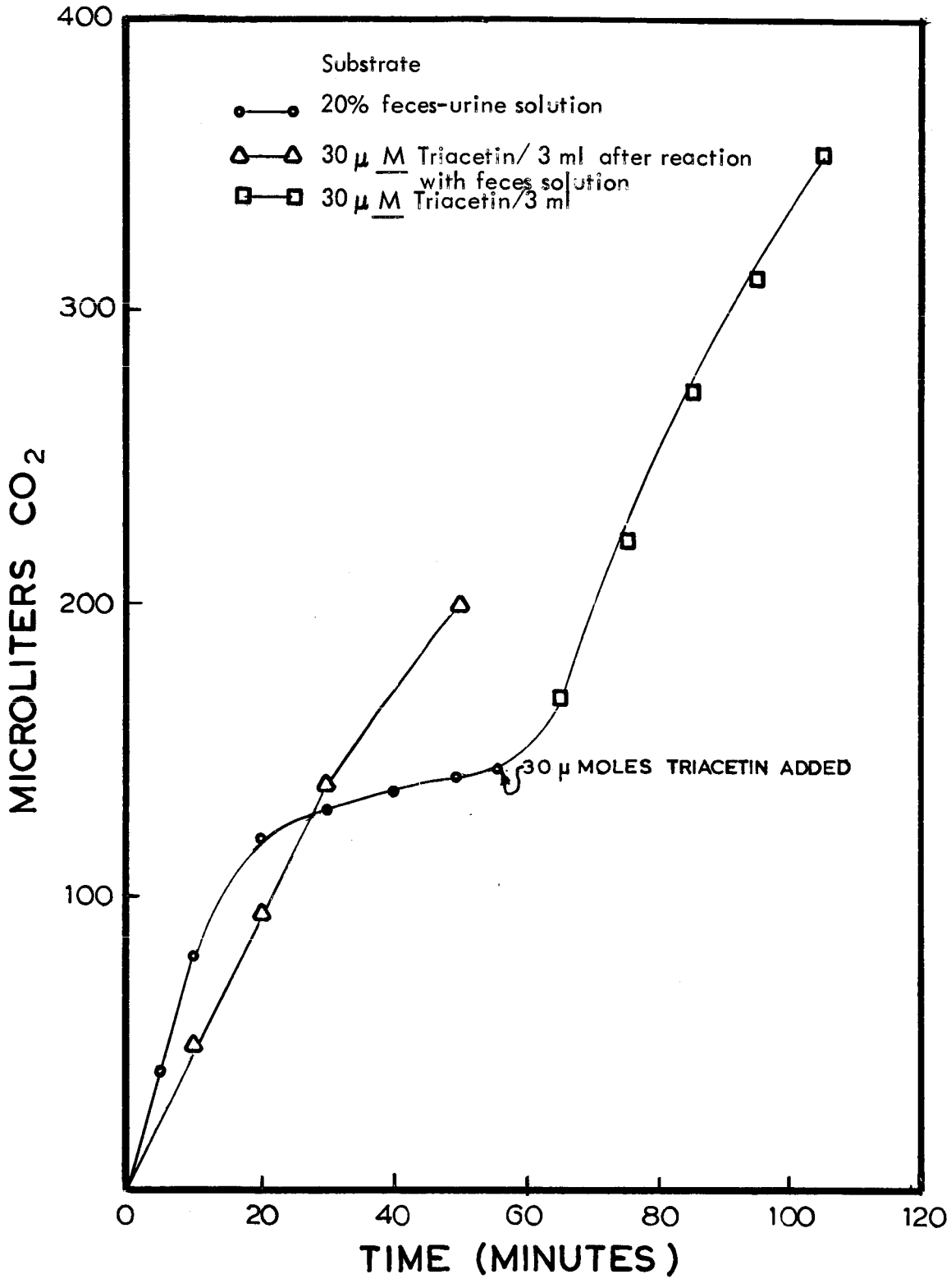

Figure 3

LIPASE ACTIVITY WITH TRIACETIN AND URINE-FECES MIXTURE

capsule waste handling cannot be fully assessed without the benefit of further research. There is little question, however, that the most logical approach to this problem involves directly applying present waste-treatment technology as the point of departure for closed ecology studies.

4.3.1 Aerobic Digestion

An aerobic microbiological system offers several advantages over that of anaerobic processes: (a) oxidation efficiencies of organic matter in the range of 95 - 98% are common; (b) no toxic gases are produced; (c) odors are rapidly eliminated; (d) the waste sludge contains useful products such as amino acids, vitamins, and minerals; and (e) temperature requirements are low. The disadvantages of an aerobic process include: (a) large requirements for oxygen; (b) large power requirements for supporting mechanical equipment; (c) large fluid volume requirement. It does not appear feasible to employ aerobic processes as a part of power-generating electrochemical processes. Attention is being given, however, to electrochemical methods through which the power requirements of aerobic digestion can be decreased significantly.

4.3.2 Anaerobic Digestion

The major advantage of an aerobic digestion process is the ability to digest high loads of wastes with low fluid volume requirements. The most severe disadvantage of this process is the production of toxic or hazardous by-products which include methane, ammonia, hydrogen sulfide, and various reduced organic compounds. Generally, anaerobic digestion is slower and less reliable than aerobic processes. In addition, optimum digestion occurs at 55 - 60°C, which increases power requirements.

An initial approach has been made to the combined problem of waste handling and power generation, and is discussed in Section 4.2.2.2 under production of hydrogen and ammonia from feces.

4.4 Experimental

4.4.1 Urine as Fuel

4.4.1.1 Determination of Gas Phase

A 1% inoculum of a 48-hour urine-grown culture of B. pasteurii was added to 50 ml of filter-sterilized urine containing 0.1% ascorbic acid. The initial pH was adjusted to 8.8. Ten ml aliquots of the inoculated urine were dispensed into three, 125 ml Warburg respirometer flasks. Mercury was used as the manometer fluid. Precautions were taken to prevent contamination. The flasks were evaluated and flushed with helium while being agitated in a 30 C water bath. After the flasks were flushed for 30 minutes, the manometers were adjusted to atmospheric pressure and the flasks were incubated for four days. Data are shown in Table I.

Urea analyses were performed, as previously described, on initial and final samples of the cultures.

The Warburg respirometers were not calibrated and, therefore, approximate K values were used based upon previous calibrations.

4.4.1.2 Effects of Urea Concentration

Experiment I

100 ml aliquots of filter-sterilized urine containing 1.4 (unsupplemented urine) and 3.1% urea and 0.1% ascorbic acid were inoculated with 1% of a 24 hour urine-grown culture of B. pasteurii. Urine was supplemented with filter-sterilized urea. The pH was adjusted to 8.5. 30 ml screw cap tubes were filled to capacity and incubated for 24 hours. Direct cell counts were made on the inoculum and on the final cultures. Initial and final urea determinations were performed and initial and final ammonia concentrations were determined by the method of Gentzkow and Masen.[4]

TABLE 1

Gas Production Demonstrated Manometrically by Cultures of Bacillus pasteurii

Flask No.	Initial Values		Final Values (4 days)		
	Urea (mg/ml)	pH	Urea (mg/ml)	pH	Approx. Gas Produced (ml of gas/10 ml of culture
1	10.75	8.8	0.29	9.3	2.0
2	10.75	8.8	0.28	9.3	2.0
3.	10.75	8.8	0.29	9.3	2.0

TABLE 2

Effect of Urea Concentration on Growth of B. pasteurii

% Urea	Average Urea Utilized mg/ml	% Urea Utilized	Average NH_3 Produced mg/ml	Average Final Cell Count	Mole Ratio Urea/NH_3
1.0[2]	9.87	96.5	5.24	3.0×10^7	1:1.87
1.0[2]	9.85	96.2	5.08	2.5×10^7	1:1.83
1.4[1]	13.48	93.8	7.22	5.9×10^7	1:1.89
1.4[1]	13.40	93.4	7.11	5.8×10^7	1:1.88
1.9[2]	17.43	89.0	9.74	2.8×10^7	1:1.69
1.9[2]	18.53	94.5	9.92	2.4×10^7	1:1.85
2.7[2]	21.06	78.8	9.92	3.5×10^7	1:1.66
2.7[2]	21.31	79.7	10.76	2.4×10^7	1:1.79
3.1[1]	20.93	68.6	9.63	4.6×10^7	1:1.62
3.1[1]	21.68	69.0	10.43	5.7×10^7	1:1.70
3.5[2]	22.37	64.6	10.65	2.4×10^7	1:1.68
3.5[2]	21.00	60.9	9.74	1.5×10^7	1:1.70
3.9[2]	22.65	57.7	10.63	1.8×10^7	1:1.71
3.9[2]	22.75	58.0	11.15	2.3×10^7	1:1.73

1 Experiment 1; cell count of inoculum - 6.1×10^7 /ml

2 Experiment 2; cell count of inoculum - 2.2×10^7/ml

Experiment 2

100 ml aliquots of filter sterilized urine containing 1.0 (unsupplemented urine) 1.9, 2.7, 3.5 and 3.9% urea and 0.1% ascorbic acid were inoculated with 1% of 24 hour urine-grown culture of B. pasteurii. Urine was supplemented with filter-sterilized urea. 30-ml screw cap tubes were filled to capacity and incubated for 24 hours. The pH was adjusted to 8.6. The same analytical procedures were used as in Experiment 1. The data of both experiments is presented in Table 2.

4.4.1.3 Optimum Temperature Study

300 ml filter-sterilized urine containing 0.1% ascorbic acid was inoculated with 1% of a 24 hour urine-grown culture of B. pasteurii. The pH was adjusted to 8.6. Duplicate 30-ml screw cap test tubes were filled to capacity with culture and incubated at 25, 30 and 35 C for 24 hours. Cell counts were made using a Petroff-Hauser counter. Urea and ammonia concentrations were determined at 24 and 48 hour intervals. The results are presented in Table 3.

4.4.1.4 Continuous Culture

The continuous culture apparatus is illustrated in Figure 4. The growth vessel was inoculated with 50 ml of a 24 hour urine-grown culture of B. pasteurii added to 50 ml of filter-sterilized urine. The pH was 8.9 and the temperature was maintained at approximately 31 C. The culture was mixed with a magnetic stirring bar placed on the bottom of the growth vessel. Samples for analysis were collected from the overflow tube. After 121 hours of continuous operation the pH of the culture was 9.2 and the total cell count was 1.2×10^8. This concentration of cells is about one order of magnitude higher than that which occurs in stationary culture. The results are summarized in Table 4.

TABLE 3

Effect of Temperature on Growth of B. pasteurii

°C	Age Culture	Ave. Urea[1] Utilized mg/ml	% Urea Utilized	Ave. NH_3 Produced mg/ml	Ave. Final Cell Count Cells/ml	Mole Ratio Urea/NH_3
25	24	4.70	38.4	1.49	2.2×10^7	1:0.114
25	24	3.30	26.9	1.91	3.6×10^7	1:0.204
25	48	10.59	86.4	5.57	-	1:1.86
25	48	10.15	84.5	5.04	-	1:1.76
30	24	11.66	95.4	6.16	4.2×10^7	1:1.88
30	24	11.72	95.6	6.16	6.2×10^7	1:1.85
30	48	12.01	98.0	5.83	-	1:1.72
30	48	12.01	98.0	6.19	-	1:1.82
35	24	11.64	95.2	6.10	3.6×10^7	1:1.85
35	24	11.72	95.6	6.20	5.2×10^7	1:1.86
35	48	12.01	98.0	6.02	-	1:1.77

1 Original urea - 12.25 mg/ml

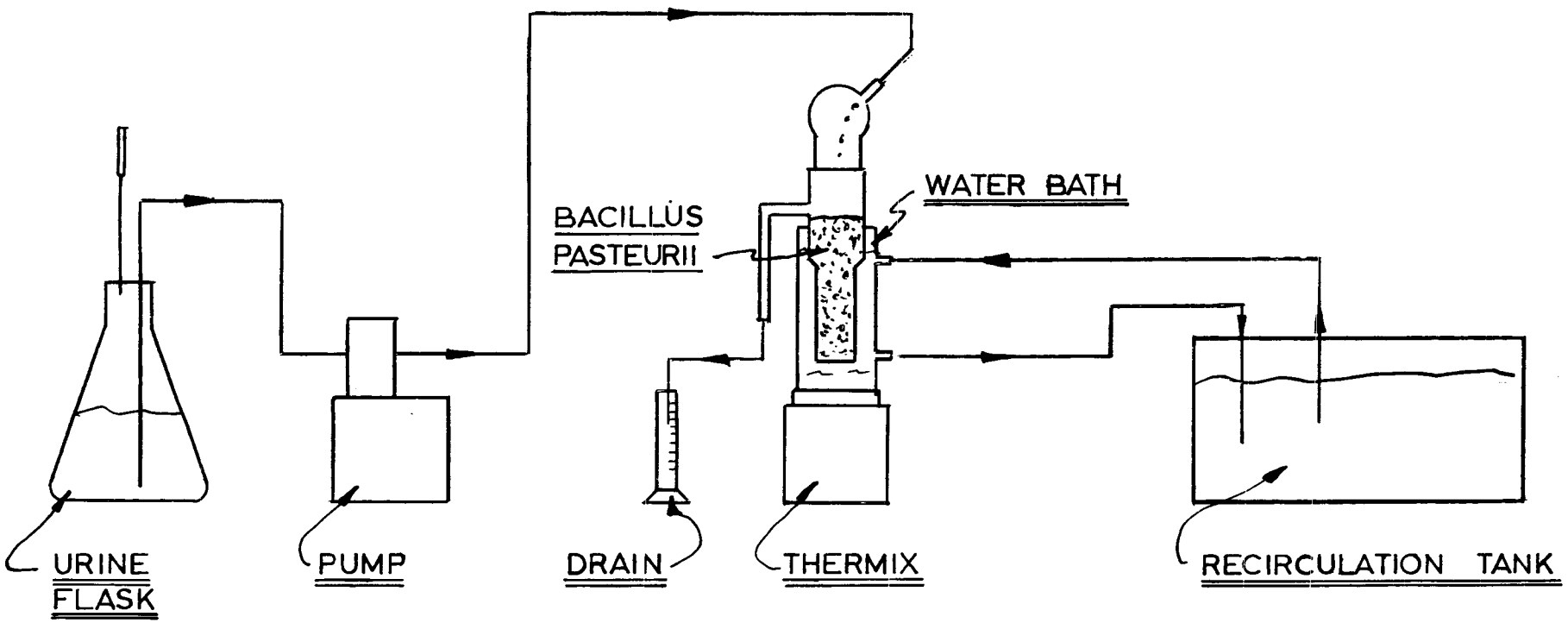

Figure 4

Continuous Culture of Bacillus pasteurii in Urine

TABLE 4

Growth of B. pasteurii in Continuous Culture

Age Culture (hrs)	Medium Flow Rate	Ave. NH_3 Produced mg/ml	Ave. Urea Utilized mg/ml	% Urea Utilized	Mole Ratio Urea/NH_3
5		2.65	4.62	38.4	1:1.99
22	19.4 ml/hr	2.96	4.93	41.0	1:2.1
29		3.23	4.50	37.5	1:2.53
46	17 ml/hr	4.52	7.50	62.5	1:2.12
51		4.26	8.00	66.5	1:1.88
121	13 ml/hr	5.34	10.8	87.0	1:1.74

4.4.2 Mixed Systems

4.4.2.1 Growth of B. pasteurii in Feces-Urine Medium

Experiment 1

Flasks containing 4, 20 and 50% frozen feces (by weight) were autoclaved for 25 minutes. 100 ml filter-sterilized urine was added to each flask and the media inoculated with 1% of a four day old urine-grown culture of B. pasteurii. The pH was adjusted to 8.0 - 8.5. Duplicate 30 ml screw cap tubes were filled to capacity with each culture and incubated at 30 C for one week. Initial and final urea determination were made on filtered samples of all cultures. Difco Heart Infusion agar and urea broth agar plates were streaked with the 4, 20, and 50% feces cultures and incubated aerobically and anaerobically. No evidence of contamination was observed. The results are summarized in Table 5.

Experiment 2

100 ml aliquots of filter-sterilized composited urine containing 0.1% ascorbic acid were added to flasks containing 1.5 and 10 g. sterile lyophilized feces. The medium was inoculated with 1% of a 24 hour urine-grown culture of B. pasteurii and the pH adjusted to 8.8. Duplicate 30 ml screw cap test tubes were filled to capacity with the cultures and incubated at 30 C for 42 hours. Control tubes contained the same medium minus feces. Original and final urea and ammonia determinations were performed. The results are given in Table 6.

4.4.2.2 Treated Sewage Experiment

Treated sewage was obtained from the primary sewage treatment plant at Huntington Beach, Calif. Three liter macro-respirometers were used as digestors in this experiment. Digestion flasks contained 200 ml aliquots of culture

TABLE 5

Growth of B. pasteurii in Feces-Urine Medium

Tube No.	% Feces by weight	Ave. Original Urea	Ave. Final Urea	% Urea Utilization	Final pH
1	4	8.0	7.59	95.0	9.2
2	4	8.0	7.61	95.2	9.2
3	20	7.5	6.52	88.0	9.1
4	20	7.5	6.70	89.5	9.1
5	50	7.0	5.62	80.3	8.8
6	50	7.0	5.62	80.3	9.0

TABLE 6

Growth of B. pasteurii in Feces-Urine Medium

% Feces by weight	Initial Urea mg/ml	Ave. Urea utilized mg/ml	% Urea Utilized	Ave. NH_3 Produced mg/ml	Mole Ratio Urea/NH_3
0	12.75	12.41	97.5	5.44	1:1.54
0	12.75	12.44	97.6	5.75	1:1.64
1	15.00	14.42	96.4	5.92	1:1.51
1	15.00	14.45	96.4	5.46	1:1.33
5	12.00	10.30	85.5	5.34	1:1.83
5	12.00	10.35	86.4	5.64	1:1.91
10	10.25	7.70	75.0	4.92	1:2.26
10	10.25	8.05	58.5	5.12	1:2.24

which were prepared from 100 ml phthalate buffer (pH 5.0), 25 ml filter-sterilized urine, 5 gm sterile, lyophilized feces (5%) and 375 ml treated sewage. Control flasks contained 200 ml aliquots of culture which were prepared from 100 ml potassium acid phthalate-NaOH buffer (pH 5.0), 25 ml sterile distilled water and 375 ml treated sewage. The initial pH of both cultures was 5.5. Flasks were evaluated and flushed 10 times with helium. Manometers were filled to capacity with acidified water. The digestors were incubated at 30 C with shaking for six days. Total and volatile solids were determined on original and final cultures. Gas evolution was measured manometrically and the gas phase analyzed using gas chromatography. The results are presented in Table 7.

4.4.3 Source of Urine and Feces

Urine and feces for this program were obtained from volunteers. Contributors were asked to consume a simulated space diet, the principle feature of which was omission of raw fruits and vegetables. Personnel on otherwise specialized diets or who were taking medications were not asked to contribute.

A total of approximately 20 gal. of urine and 30 lbs. of feces was collected. Both were frozen immediately after collection. At the close of the collection program, the urine was thawed and mixed, distributed in approximately half-gallon lots, and refrozen. The feces are presently being processed. The procedure used is to freeze-dry the feces without thawing, to break the freeze-dried material down to a powder in a Waring blendor, and to store under nitrogen in a refrigerator. When sufficient feces have been obtained, the material will be mixed and analyzed.

4.4.4 Enzyme Experiments

4.4.4.1 Dialysis of Cellulase Preparation

100 mg of the crude fungal cellulase preparation (N.B.C.) was suspended in 50 ml of distilled water. This solution was placed into cellophane

TABLE 7

Anaerobic Digestion of Feces-Urine Medium

Flask No.	5	6	7	8
(ml gas evolution at STP, days)	Control	Control	Urine + feces	Urine + feces
1	0	0	83.0	90.5
4	0	0	291.0	257.0
5	0	0	291.0	355.0
6	0	0	326.0	374.0
Final pH	6.2	6.2	5.9	5.8
Total solids g/l Original	4.01	4.01	49.07	49.07
Final	3.98	4.06	34.46	32.99
% Reduction	0.75	1.20	29.90	32.7
Volatile solids g/l Original	2.72	2.12	42.20	42.20
Final	1.54	1.56	26.87	24.51
% Reduction	27.40	26.40	36.40	42.00

dialysis tubing and allowed to remain in 0.01 M phosphate buffer, pH 7.0 at O°C for a period of 16-18 hours. The enzyme solution was removed from the dialysis tubing and diluted to 100 mls with cold distilled water.

4.4.4.2 Temperature Study with Cellulase

A 1% solution (w/v) of carboxymethylcellulose (Hercules Cellulose gum 7 HP) was diluted with 20 ml of McIlvaine buffer solution, pH 5.0 and 60 ml of distilled water. Nine ml of this solution was added to each of five tubes. Each set of five tubes was placed into a water bath of the appropriate temperature (30°C, 40°C and 50°C) and allowed to equilibrate. To each tube 1 ml of the above mentioned enzyme solution was added and the mixture was well agitated. A zero time sample (1 ml) was removed immediately from one tube and the amount of reducing sugar determined as described previously. Samples were taken every hour thereafter from a different tube. Five samples' were taken in all. The amount of reducing sugars (glucose and cellobiose) present were determined by referring to a calibration curve (O.D. vs. glucose concentration) as described previously.

4.4.4.3 Lipase Assay

The lipase assay used is a manometric method described in the literature by E. D. Wills.[5] In those experiments where triacetin (Eastman 256) served as the substrate, 0.85 ml of 1.0 M $NaHCO_3$, 0.1 or 0.05 ml of enzyme solution, and water (to make a total volume of 3.2.ml) were added to the main compartment of the Warburg flask. The triacetin (neutralized) solution (30 to 50μ M) was added to the side bulb and was tipped in to start the reaction. When the 20% feces (W V) solution (in urine) was used as the substrate, the lipase solution was tipped in to start the reaction and the feces solution (1.0 ml) was added to the main compartment along with the $NaHCO_3$ solution. In both cases, the Warburg cups

were purged for 15 minutes with 100% CO_2. This concentration of $NaHCO_3$ and the 100% CO_2 atmosphere buffered at pH 7.4. All manometric assays were carried out at 37°C.

4.4.4.4 Lipase Solution

One hundred milligrams of crude pancreatic lipase (Lipase 448-N.B.C.) was suspended in 10 ml of distilled water, placed in a cellulose dialysis bag and dialyzed against 0.01 M phosphate buffer, pH 7.0 for 18 hours. Particulate material was removed by cold (4°C) centrifugation and the enzyme solution stored at 4°C. Protein concentration was determined by the Lowry colorimetric procedure.

4.4.4.5 Feces-Urine Solution for Lipase Experiments

Twenty grams (wet weight) of feces was autoclaved (15 psi, 15 min) in the container section of a Waring blender. After cooling, 100 ml of filter sterilized urine was added and the mixture blended for 15 minutes. The pH of the mixture was adjusted to 7.4 with 0.1 M NaOH and then it was stored at 4°C in a sterile flask.

5.0 CONCLUSIONS

Estimates of electrical energy that may be derivable from urine and feces show urea in urine to be the most abundant individual potential fuel component of urine and feces. Further, urea accounts for more than 60% of the electrical energy estimated to be producible from urine and feces.

The estimates made lead to two approaches to utilizing urine-feces mixtures as electrochemical fuels:

(a) stimulation of biological ammonia production from urine by added feces, and

(b) biological production of hydrogen and/or ammonia (or other electoactive intermediates) from feces-urine mixtures using mixed bacterial cultures.

Preliminary experiments indicate that feces actually inhibits production of ammonia from urine by B. pasteurii. Based on estimated energy availability from urine, if feces cannot be shown to stimulate ammonia production from urine, it appears that urine would best be considered separated from feces as a bioelectrochemical fuel.

Work to obtain hydrogen, ammonia, or other electroactive intermediates-mixed culture action on bacterial-feces mixtures is inconclusive to date.

6.0 PROGRAM FOR NEXT INTERVAL

Intensive efforts during the coming quarter will be devoted to:

(a) establishing optimum conditions for Bacillus pasteurii conversion of urea to ammonia in urine,

(b) evaluating electrochemical performance of B. pasteurii cultures in the cell constructed during this report period, and

(c) bacterial production of hydrogen and/or ammonia from urine-feces mixtures.

With respect to optimum conditions for B. pasteurii action on urea, particular attention will be given to the concept of combining feces with urine. Electrochemical behavior is to be limited to B. pasteurii in urine until such time as feces may be shown to contribute in independent work. Urine-feces mixtures will be attacked primarily via adaptation of anaerobic cultures demonstrated to be suited for digestion of such mixtures.

7.0 REFERENCES

1. Research on Applied Bioelectrochemistry, Report No. 1, March-June 1963, Contract NASw-623, Magna Corporation.

2. Analysis of Human Fecal Components, S. A. Goldblith and E. I. Wick, Mass. Inst. of Tech. ASD. Tech. Report No. 61-419, Contract AF 33(616)-6136, August 1961, ASTIA No. AD 266882.

3. W. I. Ingram, Report No. AMRL-TDR-62-126, 1962, p 21. ASTIA No. AD 294480.

4. C. J. Gentzkow, J. Biol. Chem., 143, 531 (1942)

5. E. D. Wills, Biochem J., 57, 109 (1954)

8.0 IDENTIFICATION OF KEY PERSONNEL

Key technical personnel assigned during this quarter are as follows:

		Man hours
J. H. Canfield	Head, Life Sciences (Project Manager)	50
J. J. Cavallo	Research Biochemist	72
B. H. Goldner	Senior Research Microbiologist	221
M. D. Lechtman	Research Microbiologist	186
R. Lutwack	Senior Research Chemist	98
C. Albright	Research Chemist	346
Technicians		320